NOVEL USE OF CYTOKINE INHIBITORS

Cross-reference to related application

This application claims priority under 35 U.S.C. §§ 119 and/or 365 to Swedish Patent Application Serial No. 0200667-4 entitled "Novel Use of Cytokine Inhibitors" and filed on March 5, 2002, the entire content of which is hereby incorporated by reference in its entirety.

Field of the invention

The present invention relates to pharmaceutical compositions and methods for prevention and/or reduction of formation of scar tissue and/or formation of adhesions.

Background of the invention

In general, wound healing is a positive physiological reaction that may restore anatomy and function of various tissues after trauma. The trauma may be accidental, the result of surgical intervention or the effect of a disease or genetic condition. The ideal end result of wound healing should be to restore the tissues to the situation before the trauma. One important part of the wound healing process is to form connective tissues or scar tissue that may support the healing tissues during wound healing and regeneration. However, in many cases during wound healing, the newly formed connective tissues (scar tissue) may interfere negatively with the normal function of the healing tissues. The wound healing with formation of new connective tissues may also induce adhesions that may induce pathological conditions per se. Adhesions and scarring may also reduce the possibilities of later surgical intervention of the injured tissue if needed. Scar tissue may also induce cosmetically undesirable results such as cheloid formation. Examples of adhesions and scarring may be found virtually in any organ or tissue undergoing wound healing after trauma or surgery. Following abdominal surgery and following gynecological surgery it is not uncommon that the surgical procedure per se may induce adhesions that may both make later surgery more difficult and even induce pathological conditions such as ileus. Following spinal surgery it is common to have a situation with a dense scar formation called epidural fibrosis. This may in certain case induce significant difficulties for repeated surgery and has also been suggested to induce compression of the adjacent nerve tissue. In other

organs excessive wound healing may induce unwanted fixation of tissues and structures that may reduce function and induce pathological conditions. In general, a method for controlling the wound healing, particularly the formation of scar tissue and adhesions, would be of a great value in most cases of posttraumatic or post surgical wound healing.

In the literature it is has been recognized that foetal tissues heal with emphasis of regeneration of the injured tissue with no or little scar formation. In contrast, adult tissues instead may result in scar formation that may dominate over tissue regeneration. The fibroblasts that invade the area of wound healing have been suggested to play a key role in scar formation since they are the cells that are responsible for the formation of collagen, which is the main constituent of a scar. The fibroblasts should also play a key role in adhesion formation since the main component of adhesions is collagen formed by fibroblasts.

Since the fibroblasts are responsible for producing collagen attention has been drawn to the regulation of the fibroblasts in order to reduce scar formation. Transforming growth factor (TGF), which is an anti-inflammatory cytokine, and fibroblast growth factor (FGF) are known to stimulate the fibroblasts to produce collagen. Attempts have been made to administer a TGF-inhibitor for this purpose with varying degree of success. Tumor necrosis factor alpha (TNF) and interleukin 1 (IL-1) may reduce collagen production from fibroblasts in *in vitro* systems. However, no attempts have been made to reduce scar formation by administration of these two cytokines.

Summary of the invention

Based on the knowledge derived from the literature the inventor assessed the efficacy of inhibiting scar formation by administration of TNF in a laminectomy model on the rat (see the Comparative Example below). To his surprise, he found, contrary to what could be expected, that the wound healing was significantly impaired in the rats exposed to TNF. Scar formation and adhesions were also more common after administration of TNF compared to control.

Since administration of TNF increased scar formation and also negatively influenced the wound healing per se, the inventor realized that the *in vitro* data acquired in experimental settings regarding fibroblast regulation

are not applicable *in vivo*, and that these findings had to be re-evaluated in light of the *in vivo* situation.

The cytokine network is complex and what may seem to be evident from an *in vitro* setting may often prove not to be applicable in the *in vivo* setting. The *in vivo* situation at the area of wound healing comprises a vast number of known and unknown substances that may interact in ways not present *in vitro*. Administration of a cytokine in one concentration may have an effect that is counteracted by administration of the same cytokine in a higher dose due to synergistic inhibition and stimulation between the various cytokines as well as physiological inhibition of its release from adjacent cells.

One key mechanism essential for the formation of a scar is the physiological, inflammatory process that occurs early at the location of wound healing. The inflammation induces an increased blood flow in the wound healing area. The inflammation also induces an activation of adhesion molecules that, together with a simultaneous increase in vascular permeability, may facilitate the migration of inflammatory cells to the wound healing site. Inflammatory mediators also have leucotactic or chemotactic properties, i.e. attract white blood cells to the area of wound healing. Two important inflammatory mediators responsible for this leucotaxis are TNF and IL-1. In delayed type hypersensitivity (DTH), TNF and IL-1 augment the inflammatory reaction induced by inflammatory cells. TNF and IL-1 synergistically with other chemokines stimulate local macrophages to produce fibroblast stimulating substances that will stimulate the fibroblasts to produce collagen.

The inventor therefore assumed that a more feasible way to prevent scar formation than previously suggested was to reduce the activity of the involved substances, which he also later found to be true. This both prevent cell migration to the site of wound healing and also reduces the production of fibroblast stimulating substances. Since TNF and IL-1 are responsible for both these mechanisms, the most efficient way to reduce scar formation and adhesion is to inhibit the action of these two pro-inflammatory cytokines or other pro-inflammatory cytokines.

The characterizing features of the invention will be evident from the following description and the appended claims.

Detailed description of the invention

As discussed above, and further demonstrated in the Example below wherein administration of infliximab to rats with a standardized laminectomy is discussed, the inventor found, contrary to what could be expected from existing literature, that inhibition of pro-inflammatory cytokines is an efficient way to control wound healing and to prevent and/or reduce scar tissue formation and/or adhesion formation. Such pro-inflammatory cytokines are tumor necrosis factor (TNF), interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 12 (IL-12), interleukin 15 (IL-15), interleukin 17 (IL-17), interleukin 18 (IL-1), granulocytes-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein 1 (MIP-1), RANTES (regulated upon activation, normal T-cell expressed, and presumably secreted), epithelial cell-derived neutrophil attractant-78 (ENA-78), oncostatin-M (OSM), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF); and in particular TNF (also called TNF-α) and IL-1 (including both IL-1 α and IL-1 β). The exact mechanisms behind this are not fully known. However, the reduced scar formation may be the result of a reduced inflammatory reaction at the wound site, with reduced recruitment of inflammatory cells and fibroblasts, and by a reduced stimulation of macrophages.

The use and method according to the invention for reduction of scar formation under these conditions are extremely valuable for controlling wound healing, thereby maintaining the normal function and regeneration of the injured tissue, allowing for repeated surgery and reducing the risk of scar induces pathological conditions. The pharmaceutical composition and method according to the invention are thus suitable for treatment of posttraumatic tissue injury. Posttraumatic tissue injury may be, for example, the result of an accident. Posttraumatic tissue injury may also be caused by surgery or surgical intervention. Scar formation may also result from a pathological condition. The pathological condition may be caused by a vascular disease, such as bleeding or infarct, which may lead to necrosis. The pathological condition may also be caused by a toxic influence, such as damage caused by an acid, or by thermic injury, such as burn injury. Furthermore, the pathological condition may be of a genetic origin, such as cystic fibrosis. The end result of wound healing may also produce hypertrophic scarring, e.g. cheloid.

The suggested treatment is applicable at all kinds of surgery. It may also be used after traumatic tissue injury. Tissue injury may also be the result of toxic influence, as the result of reduced blood flow due to vascular disease, or as the result of a thermic injury, and the treatment according to the invention is applicable also for these three latter conditions. The invention is also applicable to prevent cheloid formation.

For the purpose of this disclosure, the terms "blocking agent", "blocking substance", "inhibitor" and "antagonist" may be used interchangeably.

As stated above, inhibition of a pro-inflammatory cytokine is useful for the reduction of scar formation. This inhibition is possible to achieve by any suitable cytokine inhibitor, such as available pharmacological compositions.

Persons skilled in the art are well aware of what is intended by a pro-inflammatory cytokine. For the purpose of this disclosure, it may, however, be further clarified that the expression “a pro-inflammatory cytokine” relates to any substance from the cytokine family that posses one or more of the following specific mechanisms of action: 1) increasing vascular permeability, 2) attracting white blood cells (leucotaxia or chemotaxia), 3) activating macrophages, and 4) recruiting macrophages to the site of wound healing. These effects may be assessed for each individual substance by use of the assays disclosed below. “A substance that inhibits a pro-inflammatory cytokine” as it is used herein thus relates to a substance that may block one or more of the four listed effects in the assays disclosed below. However, due to differences between species, one may also translate findings from the experimental setting to the human situation. For instance, if a monoclonal antibody with specificity towards a specific cytokine of a certain species inhibits the action of the cytokine in one of the three ways disclosed below in that specific species, one may assume that a monoclonal antibody, with specificity towards the human version of the cytokine, may inhibit this cytokine in the human situation.

<u>1) Assay for increase of vascular permeability:</u> A golden hamster, weighing 65-100 g, is anaesthetized with a mixture of Apozepam® (Diazepam 5 mg/ml Apothekarnes Laboratorium, Oslo, Norway) and Mebumal Vet® (Penthobarbital 60 mg/ml, NordVacc Vaccin AB, Malmö, Sweden) volume ratio 10:1. An initial dose of 0.3 ml is given intraperitoneally. Additional injections of 0.1-0.4 ml are administered each 30 minutes. The hamster is placed on a heated (37° C) perspex plate, and the right cheek-pouch is everted

over a translucent rubber plate and covered with plastic film in order to prevent reduction in blood flow rate due to direct exchange of oxygen. An injection of 0.3 ml of FITC-Dextran (mw 150,000, 25 mg/ml, Sigma, St Louis, USA) is made in the femoral vein for fluorescence vital microscopic observations of macromolecular extravascular leakage. Temperature and humidity is controlled by irrigation of saline at 37°C. An injection of approximately 0.02 ml of a suitable concentration of the substance to be tested is made between the two layers of the cheek-pouch using a thin injection needle (diameter 0.4 mm). The same volume of saline is performed in an adjacent part of the cheek-pouch at a distance from the other injection site sufficient to eliminate the risk of communication between the saline and the tested substance within the cheek-pouch. The injection procedures are carried out under a stereomicroscope to minimize mechanical damage to the microvessels. Microvascular reactions are studied for 60 minutes at various magnificications, using fluorescence microscopic techniques (Leitz, Wetzlar, Germany). A pro-inflammatory cytokine as defined according to the present invention induces a leakage of the fluorescent macromolecule FITC-dextran. A similar leakage should not be observed at the site injected by saline.

<u>2) Assay for leucotaxia or chemotaxia:</u> A pig, bodyweight 25-30 kg, is anaesthetized with an intramuscular injection of 20 mg/kg bodyweight of Ketalar® (ketamine 50 mg/ml, Parke-Davis, Morris Plains, New Jersey) and an intravenous injection of 4 mg/kg bodyweight of Hypnodil® (methomidate chloride 50 mg/ml, AB Leo, Helsingborg, Sweden) and 0.1 mg/kg bodyweight of Stresnil® (azaperon, 2 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). Anaesthesia is maintained by additional intravenous injections of 2 mg/kg bodyweight of Hypnodil® and 0.05 mg/kg bodyweight of Stresnil®. One ml of a fluid containing a sufficient concentration of the substance to be tested is placed, in a suitable concentration locally in its natural form, in slow-release preparations or by continuous administration by osmotic mini-pumps, in a specially designed titanium-chamber. The chamber is 5 mm high and has a diameter of 15 mm. The top could be dismounted and is perforated with 18 holes, each with a diameter of 1.4 mm. The chamber, together with one chamber with the same volume of saline, are placed subcutaneously in the lumbar region through separate incisions, with no communication between the chambers. After 7 days the pig is reanaesthetized similar to the first procedure. The chambers are harvested and the content of the chamber is placed in a test-

tube together with 1 ml of Hanks' Balanced Salt Solution (Life Technologies, Paisley, Scotland). From this suspension, 100 μl is used to wash out the chamber for remaining cells. This procedure is repeated 5 times. The test-tube is then shaken for 15 seconds. A total of 25 μl of thc suspension and 25 μl of Türk's staining medium (Sigma, St Louis, USA) are mixed and placed in a chamber of Bürker. The total number of leukocytes in each chamber is determined using light microscopy. The chamber with a pro-inflammatory cytokine as defined according to the present invention then contains significantly more white blood cells than the chamber with only saline.

3) Assay for activation of macrophages: A macrophage cell line is bought and cultured according to the description of the manufacturer. Examples of other cell lines which can be used are: DH-82 from ECACC, Salisbury, Wiltshire, Great Britain; ACC288, ACC269 or ACC416 from Deutsche Sammlung von Mikroorganismen un Zellkulturen GmbH (DSMZ); or ICLC ATL98011 from Institute of Pharmacological Sciences, Milan Italy. The cells are cultured in multiple-well culture plates. The substance to be tested is applied to the culture-wells in various concentrations. After incubation for 6-72 hours, aliquots of the culture media (25-50 μl) of the culture media are used for assays. Assays of TNF and IL-8 and nitric oxide (NO) are performed using commercially available assays and the results are compared with assays from culture media without the addition of the substance to be tested. A pro-inflammatory cytokine as defined according to the present invention induces significantly higher levels of one or more of TNF, IL-8 or NO in the culture media compared to culture media without the tested substance.

4) Assay for recruitment of macrophages to the site of wound healing: Rats are anaesthetized with a standardized combination of pentobarbital and diazepam. The skin on the back is shaved. A 3 cm long midline incision is made in the skin and in the underlying muscle. The substance to be tested is applied in a suitable concentration locally in its natural form, in slow-release preparations or by continuous administration by osmotic mini-pumps. In control experiments, the same amount and administration of saline is executed. The skin is sutured. After 1-4 weeks the rat is re-anaesthetized and the area of wound healing in the skin and in the muscle is harvested and processed for immunohistochemistry. Commercially available antibodies for macrophage specific CD-molecules (e.g., CDw17, CD23, CD25, CD26, CD64, CD68, CD69, CD71, CD74, CD 80, CD88, CD91 and CD105) are used to visualize

the presence of macrophages in the healing tissues. The number of macrophages is then found to be significantly higher in the healing tissue when exposed to the tested substance than in tissues exposed to saline control.

Inhibition of pro-inflammatory cytokines: An inhibitor of a pro-inflammatory cytokine as defined according to the present invention will reduce the effects of the pro-inflammatory cytokine in one or more of the four assays above, i.e. increase of vascular permeability, leucotaxia and activation or recruitment of macrophages, and/or it will have an inhibitory effect on the recruitment of macrophages in the assay for inhibition of recruitment of macrophages disclosed below.

5) Assay for inhibition of recruitment of macrophages to site of wound healing: Rats are anaesthetized with a standardized combination of pentobarbital and diazepam. The skin on the back is shaved. A 3 cm long midline incision is made in the skin and in the underlying muscle. The skin is sutured. The animal receives treatment by a cytokine inhibitor in a suitable concentration and form of administration. Control animals receive no treatment. After 1-4 weeks the rat is re-anaesthetized and the area of wound healing in the skin and in the muscle is harvested and processed for immunohistochemistry. Commercially available antibodies for macrophage specific CD-molecules (e.g. CDw17, CD23, CD25, CD26, CD64, CD68, CD69, CD71, CD74, CD 80, CD88, CD91 and CD105) are used to visualize the presence of macrophages in the healing tissues. The number of macrophages is then found to be significantly lower in the healing tissue after treatment with the cytokine inhibitor than in control animals.

The term "patient", as it is used herein, relates to any human or non-human mammal in need of treatment according to the invention.

The scar formation that may be prevented according to the present invention is formation of any kind of scar, such as scars caused by surgery, e.g. repeated surgery, and scars caused by traumatic tissue injury, tissue injury resulting from toxic influence, or thermic injury, or as the result of reduced blood flow due to vascular disease.

The term "treatment" used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The treatment may either be performed in an acute or in a chronic way.

There are several different types of inhibitors of pro-inflammatory cytokines that may be used according to the invention:

- Specific TNF blocking substances, such as
  - Monoclonal antibodies, e.g. infliximab, CDP-571 (Humicade™), D2E7, and CDP-870,
  - Soluble cytokine receptors, e.g. etanercept, lenercept, pegylated TNF-receptor type I, TBP-1
  - TNF-receptor antagonists
  - Antisense oligonucleotides, e.g. ISIS-104838,
- Non-specific TNF blocking substances, such as:
  - MMP inhibitors (i.e. matrix metalloproteinase inhibitors, or TACE-inhibitors, i.e., TNF-α Converting Enzyme-inhibitors)
    - Tetracyclines, for example Doxycycline, Lymecycline, Oxitetracycline, Tetracycline, Minocycline and synthetic tetracycline derivatives, such as CMT, i.e., chemically modified tetracyclines,
    - Prinomastat (AG3340)
    - Batimastat
    - Marimastat
    - KB-R7785
    - TIMP-1, TIMP-2, adTIMP-1 (adenoviral delivery of TIMP-1), adTIMP-2 (adenoviral delivery of TIMP-2)
  - Quinolones, for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin and Temafloxacin
  - Thalidomide derivates, e.g. SelCID (i.e. Selective Cytokin inhibitors), CC-1088, CDC-501, CDC-801, and Linomide (Roquininex®)
  - Lazaroids, e.g., non-glucocorticoid 21-aminosteroids such as U-74389G (16-desmethyl tirilazad) and U-74500
  - Prostaglandins; Iloprost (prostacyclin)
  - Cyclosporin
  - Pentoxifyllin derviates
  - Hydroxamic acid derivates
  - Napthopyrans

- Phosphodiesterase I, II, III, IV, and V-inhibitors, e.g., CC-1088, Ro 20-1724, rolipram, amrinone, pimobendan, vesnarinone, SB 207499 (Ariflo®)
- Melancortin agonists, e.g., HP-228

- Other TNF blocking substances, such as:
  - Lactoferrin, and peptides derived from lactoferrin such as those disclosed in WO 00/01730
  - CT3
  - ITF-2357
  - PD-168787
  - CLX-1100
  - M-PGA
  - NCS-700
  - PMS-601
  - RDP-58
  - TNF-484A
  - PCM-4
  - CBP-1011
  - SR-31747
  - AGT-1
  - Solimastat
  - CH-3697
  - NR58-3.14.3
  - RIP-3
  - Sch-23863
  - Yissum project no. 11649
  - Pharma project nos. 6181, 6019 and 4657
  - SH-636
- Specific IL-1α and IL-1β blocking substances, such as:
  - Monoclonal antibodies
  - Soluble cytokine receptors
  - IL-1 type II receptor (decoy RII)
  - Receptor antagonists; IL-1ra, (Orthogen®, Orthokin®)
  - Antisense oligonucleotides
- Non-specific IL-1α and IL-1β blocking substances, such as
  - MMP inhibitors (i.e. matrix metalloproteinase inhibitors),

- Tetracyclines, for example Doxycycline, Trovafloxacin, Lymecycline, Oxitetracycline, Tetracycline, Minocycline, and synthetic tetracycline derivatives, such as CMT, i.e. chemically modified tetracyclines;
- Prinomastat (AG3340)
- Batimastat
- Marimastat
- KB-R7785
- TIMP-1, TIMP-2, adTIMP-1, adTIMP-2

– Quinolones (chinolones), for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, Temafloxacin;
– Prostaglandins; Iloprost (prostacyclin);
– Phosphodiesterase I, II, III, IV, and V-inhibitors; CC-1088, Ro 20-1724, rolipram, amrinone, pimobendan, vesnarinone, SB 207499.

■ Specific IL-6 blocking substances, such as:
  – Monoclonal antibodies
  – Soluble cytokine receptors
  – Receptor antagonists
  – Antisense oligonucleotides
■ Non-specific IL-6 blocking substances, such as:
  – MMP inhibitors (i.e. matrix metalloproteinase inhibitors)
    - Tetracyclines, for example Doxycycline, Lymecycline, Oxitetracycline, Tetracycline, Minocycline, and synthetic tetracycline derivatives, such as CMT, i.e. chemically modified tetracyclines;
    - Prinomastat (AG3340)
    - Batimastat
    - Marimastat
    - KB-R7785
    - TIMP-1, TIMP-2, adTIMP-1, adTIMP-2
  – Quinolones (chinolones), for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, Temafloxacin,

- Prostaglandins; Iloprost (prostacyclin)
- Cyclosporin
- Pentoxifyllin derivates
- Hydroxamic acid derivates
- Phosphodiesterase I, II, III, IV, and V-inhibitors; CC-1088, Ro 20-1724, rolipram, amrinone, pimobendan, vesnarinone, SB 207499
- Melanin and melancortin agonists; HP-228

- Specific IL-8 blocking substances, such as:
  - Monoclonal antibodies
  - Soluble cytokine receptors
  - Receptor antagonists
  - Antisense oligonucleotides
- Non-specific IL-8 blocking substances, such as:
  - Quinolones (chinolones), for example Norfloxacin, Levofloxacin, Enoxacin, Sparfloxacin, Temafloxacin, Moxifloxacin, Gatifloxacin, Gemifloxacin, Grepafloxacin, Trovafloxacin, Ofloxacin, Ciprofloxacin, Pefloxacin, Lomefloxacin, Temafloxacin,
  - Thalidomide derivates, e.g. SelCID (i.e. Selective Cytokin inhibitors), such as; CC-1088, CDC-501, CDC-801 and Linomide (Roquininex®)
  - Lazaroids
  - Cyclosporin
  - Pentoxifyllin derivates.

The pharmaceutical composition according to the invention may also comprise other substances, such as an inert vehicle, or pharmaceutical acceptable adjuvants, carriers, preservatives etc., which are well known to persons skilled in the art.

The administration of the TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention should preferably be performed early after injury to limit the inflammatory reaction occurring at the wound healing site. The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention is administered once or repeatedly until the desired result is obtained. The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention is administered in a therapeutically effective amount, i.e. an amount that will lead to the desired therapeutical effect and thus lead to an improvement of the patient's condition.

The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention may be administered in any efficacious way normally used to administer such substances. Thus, the administration may be done both systemically and locally and may be performed before, during and/or after all kind of surgical or traumatic tissue injury. The suggested treatment may also be applicable at tissue injury as the result of pathological conditions including vascular disease and toxic influence. The TNF-inhibitor and/or IL-1 inhibitor and/or pharmaceutical composition according to the invention may for example be injected via intra-articular, intravenous (i.v.), intramuscular (i.m.), intraperitoneal (i.p.), intrathecal (i.t.), epidural, intracerebroventricular (i.c.v.) or subcutaneous (s.c.) routes by bolus injections or by continuous infusion. They may also be administered orally (per os), e.g. in the form of oral preparations, such as pills, syrups, or lozenges. Furthermore, they may be administered by inhalation or intranasally. Moreover, they may be administered transepidermally, e.g. in the form of topical preparations such as lotions, gels, sprays, ointments or patches. They may also be administered in an irrigation solution or by localized injection. Finally, they may also be administered by genetical engineering.

According to one preferred embodiment of the invention, the pharmaceutical composition is formulated as a sustained-release preparation. The substance according to the invention may then, for example, be encapsulated in a slowly-dissolving biocompatible polymer.

Examples of suitable doses for different administration routes are given below. Additional dosages would be apparent to the artisan of ordinary skill. The provided dosages include any range in between as can be determined on a case-by-case basis.

Per os	10-300 mg	
i.m.	25-100 mg	
i.v.	2.5-25 mg	
i.t.	0.1-25 mg	daily to every 3rd month
inhalation	0.2-40 mg	
transepidermally	10-100 mg	
intranasally	0.1-10 mg	
s.c.	5-10 mg	
i.c.v.	0.1-25 mg	daily to every 3rd month

epidurally 1-100 mg

Examples of suitable doses for different TNF inhibitors are given below.

Drug	Preferred Dosage	More Preferred Dosage	Most Preferred Dosage
Lenercept			
i.v.	5-200	10-100	30-80
(all doses given in mg for administration once every 4th week)			
TBP-1			
i.v.	5-200	10-100	30-80
(all doses given in mg for administration once every 4th week)			
CDP-571 Humicade®			
i.v.	1-100	5-10	5-10
(all doses given in mg/kg body weight for administration as a single dose)			
D2E7			
i.v.	0.1-50	0.5-10	1-10
s.c.	0.1-50	0.5-10	1-10
(all doses given in mg/kg body weight for administration as a single dose			
Iloprost			
i.v.	0.1-2000	1-1500	100-1000
(all doses given in μg/kg body weight/day)			
intranasally	50-250	100-150	100-150
(all doses given in μg/day)			
CC-1088			
Per os	50-1200	200-800	400-600
(all doses given in mg/day)			
CDP-870			
i.v.	1-50	2-10	3-8
(all doses given in mg/kg body weight for administration once every 4th week)			
s.c.	50-600	100-400	100-200
(all doses given in mg/day)			
Linomide			

Drug	Preferred Dosage	More Preferred Dosage	Most Preferred Dosage
Roquinimex®			
Per os	0.1-25	5-20	10-15
(all doses given in mg/kg body weight/day)			
HP-228			
i.v.	5-100	10-50	20-40
(all doses given in μg/kg body weight)			
ISIS-104838			
Per os	1-100	10-50	20-50
s.c.	1-100	10-50	20-50
i.v.	1-100	10-50	20-50
(all doses given in mg)			
Ariflo® SB 207499			
Per os	10-100	30-60	30-45
(all doses given in mg/day)			
KB-R7785			
s.c.	100-500	100-300	100-250
(all doses given in mg/kg body weight/day)			
Prinomastat (AG3340)			
Per os	1-250	5-100	10-50
(all doses given in mg for administration twice daily)			
Batimastat			
Per os	1-250	5-100	10-50
(all doses given in mg for administration twice daily)			
Marimastat			
Per os	1-250	5-100	10-50
(all doses given in mg for administration twice daily)			
CDC-501			
Per os	50-1200	200-800	400-600
(all doses given in mg/day)			
CDC-801			
Per os	50-1200	200-800	400-600

Drug	Preferred Dosage	More Preferred Dosage	Most Preferred Dosage
(all doses given in mg/day)			

It is possible to use either one or two or more substances according to the invention in the prevention of scar formation. When two or more substances are used they may be administered either simultaneously or separately.

The substances according to the invention may also be administered in combination with other drugs or compounds, provided that these other drugs or compounds do not eliminate the effects desired according to the present invention, i.e. the effect on TNF.

It is understood that the response by individual patients to the substances according to the invention or combination therapies, may vary, and the most efficacious combination of drugs for each patient will be determined by the physician in charge.

The invention is further illustrated in the Example below, which is only intended to illustrate the invention and should in no way be considered to limit the scope of the invention. The invention is also compared to the stated of the art in the Comparative Example.

Example

Four rats were anaesthetized with a standardized combination of pentobarbital and diazepam. The skin on the back was shaved. Through a midline incision, a laminectomy of the 4th lumbar vertebra was performed. The spinal muscles and the skin was sutured. Two rats received an intraperitoneal injection of 4 mg/kg of infliximab. Infliximab is a monoclonal antibody towards TNF with an inhibiting action with a duration of 1-2 months following single administration in the dosage used. The other two rats received an intraperitoneal injection of saline. After 2 weeks, the rats were reanaesthesized and wound healing and scar formation were evaluated by a person being unaware of the experimental protocol. Wound healing was considered normal in both groups. In the group of animals that received infliximab, the previous location of the lamina was filled with soft connective tissue that did not adhere to the spinal dura mater and was easy to remove. In the non-treated group,

there was a somewhat more dense lump of connective tissue that was adherent to the spinal dura mater.

Comparative example (not according to the invention)

Following a laminectomy of the lamina of the 4th lumbar vertebra either 0.15 ml of 20 ng/ml of recombinant rat TNF in distilled water or just 0.15 ml of distilled water was instilled in the laminectomy space. The wound was sutured and assessed after 1 week, 2 weeks regarding wound healing and scar tissue formation. There were 20 rats in total. Five rats were treated with TNF and five rats with only distilled water for each duration. Contrary to what could be expected the wound healing was significantly impaired in the rats exposed to TNF. The scar formation in the laminectomy space was significantly more pronounced in the TNF exposed rats, also contrary to what could be expected from the literature. The scar in the TNF exposed rats was also attached to the dura mater covering the spinal cord by adhesions. All observations were performed in a blinded fashion.